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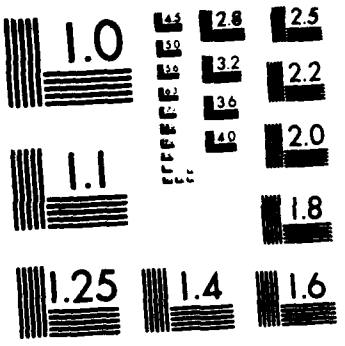

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TRIBUTYLTIN EFFECTS ON JUVENILE MUSSEL GROWTH

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ABSTRACT

Juvenile mussels (Mytilus edulis <20 mm) were exposed to three concentrations of tributyltin (TBT) in two site-specific, flow-through bioassays with unfiltered seawater. Mean TBT concentrations were 70, 80 and 200 ng/l in Test I (196 days) and 40, 50 and 160 ng/l in Test II (56 days). Treatments did not significantly affect juvenile mussel growth during the first 56 days of exposure in either test. After 63 days, all treatments significantly reduced growth in Test I. No significant mortalities occurred at any TBT concentration in either test. Increases in weights and lengths of Tank Control animals in Test II were much greater than during the first 56 days of Test I. Further, weight increases in the Pier Control were almost four times greater than in the Tank Controls during Test II. These data suggest that test animals were probably under significant stress induced by the bioassay test system. The data also suggest that the effects of TBT on juvenile mussel growth may have been overestimated in this and other studies.

INTRODUCTION

Growth represents the integrated response of internal biological process. It is generally believed that in any environment the added stress of toxicants reduces animal growth rates and that juveniles are more sensitive than adults. Significant reductions in growth rate could adversely affect the population (1). A good measure of stress in juvenile mussels is shell growth since it is a significant part of total somatic production (2) and there is no interference by gametogenesis (3).

A number of investigators have studied the effects of tributyltin (TBT) on mussel (Mytilus edulis) growth. Several laboratory studies (4, 5, 6) and a single field study (7) have reported reduced mussel growth at TBT concentrations of 230 ng/l and greater in tests ranging from 7 days to 5 months. The interpretation and environmental significance of these data are unclear (8). First, these high concentrations are not characteristic of most harbor environments and are restricted to enclosed basins with poor tidal exchange and large numbers of organotin-painted vessels (9). Second, mussel growth in the field may be different than in laboratory studies with associated growth-inhibiting stresses. Third, reported reductions in mussel growth rates may be attributable to stress from unmeasured or unknown factors (10).

Extrapolation to the environment is difficult (8, 10, 11, 12) due to differences in conditions between the above laboratory studies with TBT (4, 5) and the field. The above field study (7) provided environmentally realistic test conditions, yet still failed to confirm a cause-and-effect relationship between TBT and mussel growth rate because of unmeasured variables, uncontrolled dosing, and an inappropriate control site. Limited sample sizes and short-term exposures in previous laboratory and field studies only permit estimating order-of-magnitude effects (10) on growth of mussels exposed to high TBT concentrations. These studies have not adequately assessed the subtle effects of realistic exposure states for low TBT concentrations of interest (13) near those predicted to be safe (50 ng/l) in the estuarine environment (14).

Two site-specific, flow-through bioassays with TBT were conducted in San Diego Bay using a Portable Environmental Test System (PETS) to evaluate the long-term effects of low TBT concentrations on juvenile mussel growth. This approach combined the advantages of controlled laboratory dosing with realistic field test conditions in an attempt to provide more meaningful results than previous studies. This report addresses results of those site-specific bioassays and their significance in relation to previous laboratory and field studies.

METHODS AND MATERIALS

PETS was evaluated over a 7-month period in San Diego Bay using TBT leachates. A more detailed description of the test site and the physical/chemical parameters monitored are presented elsewhere (15). As part of that evaluation, two overlapping tests were conducted with juvenile mussels (M. edulis). Test I lasted 196 days (June to December 1986). Test II lasted 56 days (October to December 1986). Test II was conducted concurrently with the last 56 days of Test I. During that time mussels from each test were in the same tanks and subjected to identical experimental conditions. Temperatures in PETS tanks ranged from 15.0 -

25.9°C($\bar{x}$ = 22.3°C) in Test I and 15.0 - 21.7°C ($\bar{x}$ = 18.6°C) in Test II. Bay temperatures at the seawater intake ranged from 14.0 - 24.9°C ($\bar{x}$ = 21.9°C) during Test I and 14.0 - 20.8°C ($\bar{x}$ = 17.8°C) during Test II. In a single 24-hour study in December, temperature ranged from 13.5 - 16.9° in PET tanks and 15 - 16 C° at the seawater intake.

The experimental design consisted of a control and three TBT test concentrations with three replicates each and approximately 50 animals per treatment. Mean TBT concentrations (±s.d.) were 70 (±40), 80 (±40) and 200 (±70) ng/l in Test I and 40 (±15), 50 (±19) and 160 (±66) ng/l in Test II. These concentrations represented nominal 10, 25 and 100% leachate solutions, respectively, but measured concentrations were markedly different than expected (15). Mean TBT concentration in control seawater was approximately 10 ng/l. TBT concentrations were measured by hydride derivatization and atomic absorption detection (5) and reported as tributyltin chloride. All plastic holding trays were leached for 2 weeks in the laboratory with filtered flow-through seawater. Mussels were initially selected by length (~10-15 mm), taking great care to randomly distribute them within the replicate plastic holding trays. There were no significant differences in weights or lengths among replicates at the start of either test. All test animals were acclimated for 2 weeks in PETS control tanks before the experiment began.

Test I began with 192 juvenile mussels, 16 animals per test tank (48 per treatment). Some animals died and some escaped; the 163 survivors had initial lengths of 10.7 - 17.0 mm ($\bar{x}$ = 14.4 mm) and weights of 124 - 563 mg ($\bar{x}$ = 313 mg). Mussels were collected from plexiglas panels that were suspended at the test site in January. This was done to ensure that all test animals were from the same spawning season and were approximately the same age.

Test II began with 234 mussels, 18 animals per tank (54 per treatment) and 18 for the Pier Control. One mussel escaped; the 233 survivors had initial lengths of 10.1 - 15.0 mm ($\bar{x}$ = 12.6 mm) and weights of 142 - 553 mg ($\bar{x}$ = 287 mg). The purpose of the Pier Control was to determine if the test system affected growth. Without sufficient numbers of mussels from the same site, Test II animals were collected from the rubber tire fenders on the Coronado Bay Bridge approximately 1 km from the test site.

Whole-animal wet weights and lengths were measured weekly using vernier calipers and an electronic balance. Byssal threads were carefully broken prior to removing mussels from the trays for measurements. Presence/absence of byssal threads was recorded weekly as another measure of environmental stress (16, 17, 18).

Statistical analyses were conducted only on survivor data. For each treatment cumulative percent increases in lengths and weights were calculated to normalize size effects and to estimate relative growth rates for graphical presentation. Serial ANOVAs ($P < 0.05$) were performed on pooled weight and length data among replicate TBT concentrations at each sampling interval to test the null hypothesis: TBT exposure has no effect on juvenile mussel growth. If the null hypothesis was rejected, Duncan's new multiple-range test was used to determine which TBT concentrations significantly affected growth. In addition, a series of linear regression analyses were performed on log-transformed data to compare the slopes of estimated growth rates. If slopes differed by more than two standard deviations ($P < 0.05$), growth rates were considered significantly different.

RESULTS

Growth rate estimates from changes in mussel weights and lengths over time are given in Figure 1 and Table 1. Figure 2 gives the slopes of regressions performed on log-transformed 196-day weight and length data from Test I. In Test I significant reductions in growth were found between 63-196 days at all three TBT concentrations. Growth was increasingly suppressed with increasing TBT concentration. At the highest concentration mussel growth rate was approximately half the Tank Control rate. After 196 days, mussel weights and lengths in the Tank Controls and the 200 ng/l TBT treatments increased by 450% and 250%, and 65% and 37%, respectively. Mussel weights in the two lowest TBT treatments increased by 355% and lengths by 53%. Both the multiple-range test on weekly length and weight measurements and the linear regression analyses on the 196-day growth rates gave the following results: Control ≠ (70 = 80) ≠ 200 ng/l TBT treatments. That is, control growth was significantly different than treatment growth at all TBT concentrations. There was no significant difference in growth between the two lowest concentrations. Serial ANOVAs showed there was also a significant difference among treatment replicates.

In Test II there were no significant differences in lengths, weights or growth rates when TBT treatments were compared to Tank Controls. After 56 days, weights of Tank Controls and the 160 ng/l TBT treatments increased by 99% and 71%, respectively. Lengths increased by 30% and 18%, respectively. At the highest TBT concentration growth rate was approximately 70% of the control. There were significant differences in weights, lengths and growth rates between the Pier Control and Tank Controls. Pier Control mussels increased in weight by 378% and length by 73% after 56 days. In the Pier Controls and Tank Controls respectively, growth rates by weight were 140 and 40 mg/wk, and by length were 1.13 and 0.48 mm/wk. After 56 days, weights for Tank Controls in Test I and Test II increased by 70% and 99%, respectively.

In Test I, byssal thread production decreased to a minimum by day 49, when half of the mussels exposed to the highest TBT concentration produced no byssal threads. Byssal thread production remained suppressed until day 140. From then on there were no observable differences in byssal thread production at any concentration. No differences in byssal thread production were observed in Test II.

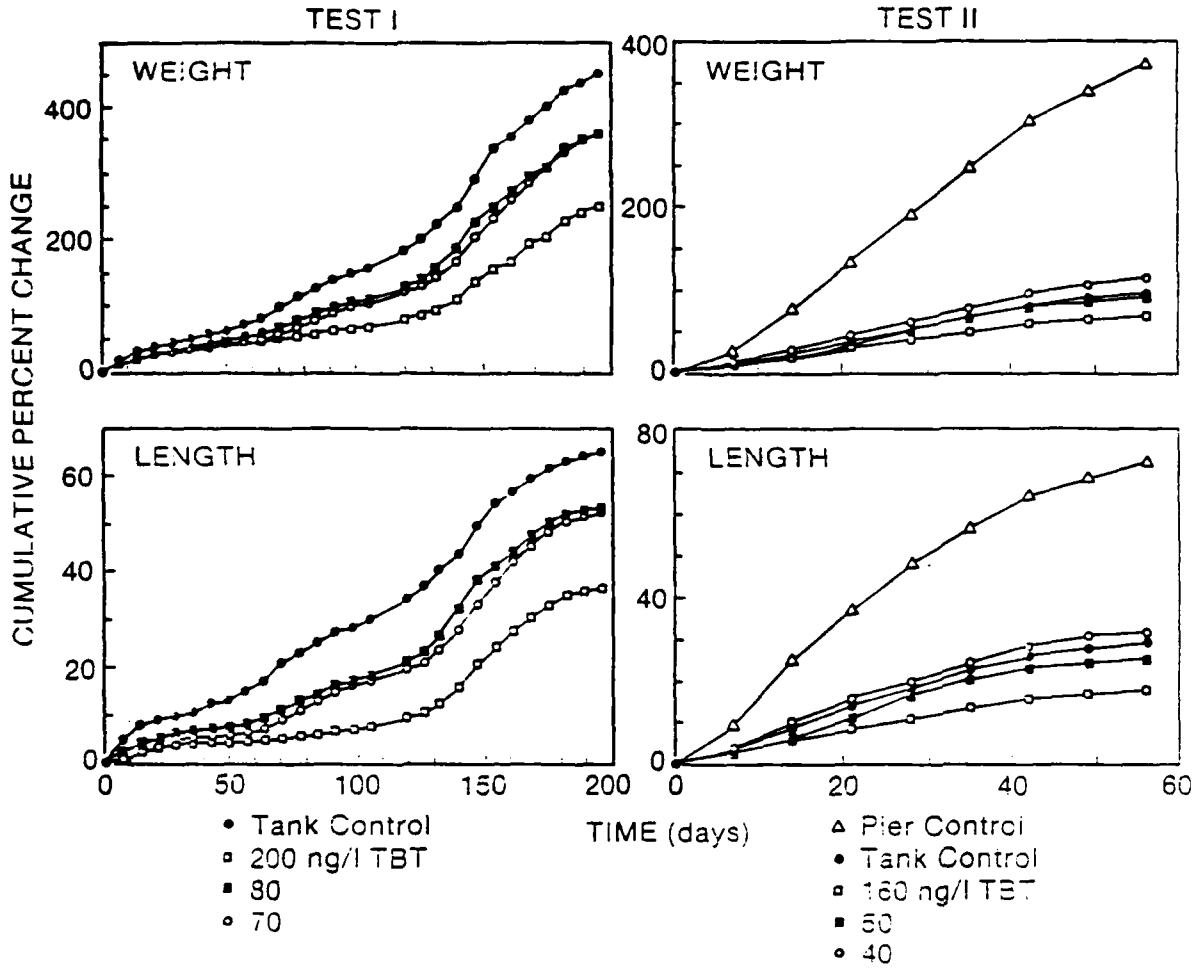

Figure 1. Cumulative percent change in juvenile mussel weights and lengths for Test I and Test II.

There were no significant mortalities at any concentration in either experiment. In Test I after 56 and 196 days exposure to 200 ng/l TBT, survival was 100 and 94%, respectively. In Test II, after 56 days exposure to 160 ng/l TBT survival was 100%.

DISCUSSION

Compared to controls, significant reductions in juvenile mussel growth rate were measured in PETS after 196 days at TBT concentrations much lower than previously reported in other laboratory or field studies (4, 5, 6, 7). No significant effects were measured after 56 days. The principal reasons for detecting these subtle effects were longer exposures and larger sample sizes than in previous studies. Since the site-specific bioassay performed here may have combined the advantages of controlled laboratory dosing with realistic field test conditions in a relatively long-term (5 month) chronic study, these results may not be surprising. PETS provided more environmentally realistic test conditions than the laboratory studies cited here because of unfiltered seawater with natural phytoplankton populations and suspended sediment. However, differences in growth rates between the Pier Control and the Tank Control suggest that test conditions were not as environmentally realistic as expected due to system-induced stress. Differences in growth between Test I and II during the first 56 days of exposure indicate that test conditions were markedly different. Therefore, the TBT concentration affecting growth in Test I may be overestimated.

Table 1. Growth rates estimated from juvenile mussel weights and lengths.

TEST I

	Pier Control	Tank Control	MEAN TBT CONCENTRATIONS (ng/l) 70	80	200
Initial Weight (mg)		319	299	330	303
Final Weight (mg)		1657	1285	1430	1005
Growth Rate (mg/wk)		48	35	39	25
0- 56 days (mg/wk)		27	14	20	15
56-119 days		42	24	26	11
119-196 days		72	59	65	44
Initial Length (mm)		14.52	14.20	14.68	14.26
Final Length (mm)		23.30	21.50	22.30	19.40
Growth Rate (mm/wk)		0.33	0.26	0.27	0.18

*

TEST II

	Pier Control	Tank Control	40	50	160
Initial Weight (mg)	293	302	278	284	283
Final Weight (mg)	1262	583	591	530	477
Growth Rate (mg/wk)	140	40	45	35	28
Initial Length (mm)	12.72	12.30	12.50	12.50	12.60
Final Length (mm)	21.76	16.60	16.50	15.70	14.80
Growth Rate (mm/wk)	1.13	0.48	0.50	0.40	0.28

* TBT concentrations were slightly different during these periods.

Test Conditions

As White and Champ (10) have suggested, some of the differences in results can be attributed to system-induced stress and differences in test conditions. Salazar et al. (15) have discussed temperature and nutritive stresses in the PETS system and suggested that tank conditions were more favorable for mussel growth in Test II than Test I. This was due to lower mean TBT concentrations, more optimum temperatures and reduced biomass during the last 56 days when the tests overlapped. Growth rates and byssal thread production of Test I mussels increased dramatically during this period. Bayne and Thompson (19) have described some of the physiological consequences of maintaining M. edulis in the laboratory as well as the specific effects of temperature and nutritive stress on reducing both growth rate and reproduction effectiveness (20, 21).

Temperature Stress

Even though experimental conditions were improved, reduced growth rates were expected in Test II since winter conditions generally reduce mussel growth due to lower temperatures and less phytoplankton (22, 23). However, growth rates of Tank Control animals were higher in Test II than the first 56 days of Test I.

Bayne et al. (1) have reported that above 20°C there is increased respiration and reduced filtration in mussels. Above 25°C there are adverse growth effects. A maximum temperature of 25.9°C and a mean temperature of 22.3°C during the early part of Test I suggests that juvenile mussels were under temperature stress. When temperature decreased in the latter part of Test I, growth rates increased in all treatments. San Diego Bay summer temperatures may reduce growth because they approach levels that adversely affect mussel physiology. Adverse temperature effects were aggravated by PET tanks which raised the temperature higher than ambient. Measured daily fluctuation was almost four times higher in PET tanks than in the bay.

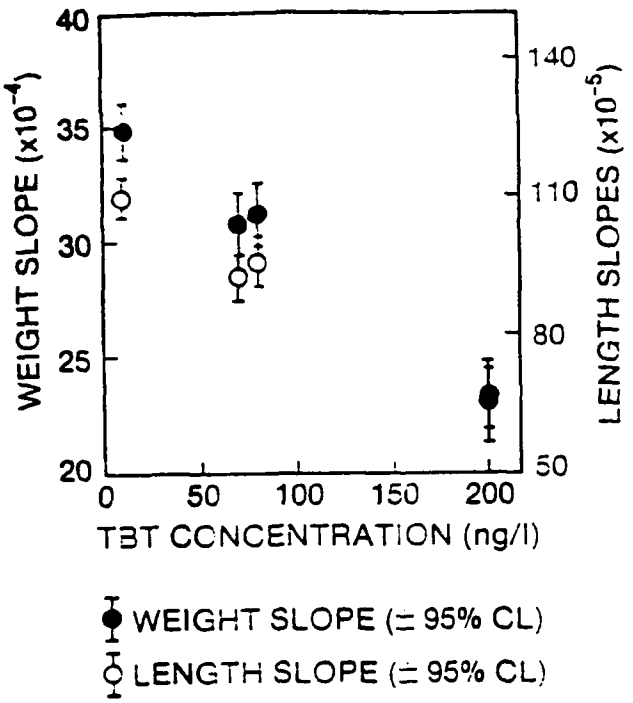

Figure 2. Log transformed weight and length slopes for juvenile mussel growth in Test I.

Nutritive Stress

The major contributing factors to nutritive stress in PETS mussels were probably reduced phytoplankton levels and reduced suspended sediment compared to ambient bay water. Weekly maintenance revealed large amounts of sediment trapped in the plumbing that never reached test tanks. As evidenced by accumulated sediment in the bottom of the PETS tanks however, there was much more suspended sediment in PETS than in laboratory studies.

Kiorboe and Mohlenberg (24) suggested growth rates in optimum laboratory studies do not approach growth rates in the field primarily because M. edulis derives additional nutrition from suspended particulates. These authors predicted growth rate increases of 30 - 70% with the addition of only 5 mg/l suspended sediment. Waldock and Thain (25) provided additional data showing a 72% enhancement in oyster growth with 75 mg/l suspended sediment. None of the previous laboratory growth studies with mussels exposed to TBT included suspended sediment. This may have resulted in nutritive stress. Mussels in any environment with suspended sediment and a natural diet may be under less total stress, grow faster and potentially more resistant to TBT.

Laboratory Studies

At test concentrations which produced no effects in PETS, Thain and Waldock (4) found a significant reduction in juvenile mussel growth. These growth reductions could be attributed to higher and more variable TBT concentrations than in PETS, but there is reason to believe that the results reflect the effects of uncontrolled test conditions (10) as much as the TBT concentrations. These conditions include using a single algal species for food, carrier solvents and no suspended sediment.

Laboratory test conditions affect growth in other ways. Widdows et al. (26) have shown reduced mussel growth with increased tissue concentrations of metals and hydrocarbons in the field. Waldock and Thain (25) have provided data that show reduced oyster growth with increasing tissue concentrations of TBT in the laboratory. Laughlin et al. (27) have shown that accumulation of TBT by mussels in the laboratory is different from that in the field. If accumulated TBT affects mussel growth rate, these results suggest that growth rates of mussels exposed to TBT in the laboratory would be different than in the field.

Laboratory studies have generally been used to estimate TBT effects on mortality and growth. Valkirs et al. (5) reported a significant decrease in length for adult mussels exposed to 300 ng/l TBT with no significant changes in weight. However, these decreases are probably an artifact of statistical analyses. They also reported a 66-day LC-10 of approximately 125 ng/l TBT for adult mussels. There were no significant mortalities attributable to TBT exposure in either Test I or II of the PETS studies at concentrations up to 200 ng/l. TBT effects on survival and growth may have been overestimated in the Valkirs et al. (5) and other laboratory tests due to nutritive stress.

Stromgren and Bongard (6) used juveniles much smaller than in PETS and reported significant reductions in mussel shell growth after only 7 days exposure to 400 ng/l TBT. While the laser measurement technique is interesting, reporting effects at such high levels in a test of such short duration with unmeasured treatment concentrations has little environmental significance.

Field Studies

Field measurements provide a realistic test platform for long-term studies, but generally lack the control necessary for experimentation and establishing cause-and-effect relationships particularly with TBT (12). In a San Diego Bay field test Stephenson et al. (7) exposed mussels at four sites along a known TBT concentration gradient of -2 km. Significant differences in growth were observed after 150 days exposure to 230 ng/l TBT. The control site, however, was inappropriate in that it differed in many parameters other than TBT concentration. In addition, there were many other variables along that TBT gradient which may have affected mussel growth. Since mussel growth can exhibit extreme local variation (22, 28, 29), the utility of using mussel growth as an index of mussel stress at different sites in the field without appropriate experimental control must be challenged. White (30) has cautioned against the arbitrary use of mussel monitoring systems without developing the model to be tested

Summary - PETS Study

Although the PETS did not duplicate the environment, it may have simulated more environmentally realistic test conditions than the laboratory because of unfiltered seawater, natural phytoplankton populations and suspended sediment. The leachate dosing system permitted greater experimental control than the field test. However, long-term exposures and large sample sizes permitted detection of significant TBT effects at concentrations much lower than previously reported. It must be emphasized that system-induced stresses also reduce juvenile mussel growth. It was impossible to quantify the relative effects of each. The 70 ng/l TBT that reduced growth rates in this study would not have an effect on mussel growth rates under most environmental conditions . Only under very stressful environmental conditions similar to those in PETS experimental tanks would this concentration affect growth. Further, 160 ng/l might have been the lowest concentration to reduce growth in PETS tanks after longer exposures and less stressful conditions. Uncontrolled stress in the site-specific bioassay precluded direct environmental extrapolation. The lowest TBT concentration affecting juvenile mussel growth under varying environmental conditions remains unknown.

The authors feel that results from the three laboratory studies, the field study and this site-specific bioassay were as much a function of uncontrolled test conditions and animal age as TBT exposure concentrations. To obtain meaningful biological measurements of TBT effects on juvenile mussel growth, the authors suggest combining laboratory, microcosm and field tests in more discriminating experiments designed to equate measured responses to the natural field response and answer specific questions about bioavailability. Further, to assess environmental significance, a leachate dosing system with TBT-coated panels similar to that used in the laboratory and in PETS should be developed for field use (in-situ). Theoretically, mussel growth rates could be used to compare dosed versus undosed animals with a control group close enough to be a true control in all other environmental parameters yet far enough away to be unaffected by TBT.

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